

MINIMAL MODEL FOR MEMBRANE OSCILLATIONS IN THE PANCREATIC β -CELL

TERESA REE CHAY

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

JOEL KEIZER

Department of Chemistry, University of California, Davis, Davis, California 95616

ABSTRACT Following the experimental findings of Atwater et al. (*In Biochemistry Biophysics of the Pancreatic β -Cell*, George Thieme Verlag, New York, 100–107), we have formulated a mathematical model for ionic and electrical events that take place in pancreatic β -cells. Our formulation incorporates a Hodgkin-Huxley type gating mechanism for Ca^{2+} and K^{+} channels, in addition to Ca^{2+} gated K^{+} -channels. Consistent with the experimental observations, our model generates spikes and bursts in β -cell membrane potentials and gives the correct responses to additions of glucose, quinine, and tetraethylammonium ions. The response of the oscillations to ouabain and changing concentrations of external K^{+} can be incorporated into the present model, although a more complete treatment would require inclusion of the $\text{Na}^{+}/\text{K}^{+}$ pump.

INTRODUCTION

A wide variety of secretory cells display oscillations in membrane potential. Among these, the β -cell is of particular interest because it involves a link between cell metabolism and membrane conductance. Glucose and other metabolizable sugars, which elicit insulin release from the β -cell of the pancreatic islet, induce repetitive oscillations in the β -cell transmembrane potential. Rapid fluctuations in potential, i.e., action potentials or spikes, occur as burst of electrical activity on each phasic depolarization. These action potentials have been associated with the stimulus for the release of insulin. The main action of glucose is to decrease the intracellular level of calcium, which is then responsible for triggering the release of insulin by exocytosis (1). A characteristic feature of β -cells is that the duration and frequency of each burst can be altered by the glucose concentration and by the external Ca^{+2} , K^{+} , and Na^{+} concentrations. In addition, mitochondrial inhibitors and uncouplers, which are known to induce a release of intracellular Ca^{+2} , block glucose-induced electrical activity (1).

Based on these experimental observation, Atwater et al. (1) have proposed a qualitative model to explain the oscillations in the β -cell. Their model includes (a) a potassium channel activated by intracellular calcium ions, (b) a voltage-gated K^{+} channel, (c) a voltage-gated calcium channel, and (d) cytoplasmic changes of intracellular calcium concentration that depend on glucose concentration.

The experimental evidence seems to us sufficiently detailed to justify construction of a dynamic mathematical

model. In this paper, we present a simple mathematical model of β -cell oscillations and look for quantitative explanations of how oscillations can arise from the interaction of membrane processes with intracellular calcium. Although our model does not include all the effects that have been observed on β -oscillations, it does include the basic features proposed by Atwater et al. (1). Furthermore, additional transport mechanisms such as the $\text{Na}^{+}/\text{K}^{+}$ pump can be added to the mechanism to account for other observed effects. In this sense, the mathematical model presented here is a minimal model, i.e., the simplest that will explain the burst pattern of β -cell oscillations.

MODEL

The model described in this section is based on recent experimental work characterizing the electrophysiology of isolated single β -cells (1–6). Using chemical blocking agents, such as quinine and tetraethylammonium ions (TEA), two sorts of potassium ion channels have been uncovered in the membrane of the β -cells. The TEA-sensitive channel appears to be similar to the voltage-gated K^{+} channel known in nerve cells (7). The channel blocked by quinine, on the other hand, is regulated by internal calcium. It appears to be related to the calcium-activated K^{+} channel found in red blood cell membranes and other tissues (8). Using these and other observations, Atwater et al. (5) proposed that calcium ion is a control agent for the membrane potential in β -cells and is responsible for the burst pattern of oscillation.

Voltage oscillations with bursts, similar to those in the β -cell, have also been observed in certain nerve cells, e.g., the R15 cell of *Aplysia* (9, 10). Recently, Plant (11–13) has explained the bursting pattern using a calcium-activated potassium conductance. An inward Na^{+} current seems to play a crucial part in these neuronal oscillations. Indeed, when the Na^{+} channel of the R15 cell is blocked by application of tetrodotoxin (TTX) the action potential spikes are abolished and only a slow oscillation waveform remains. While the effect of TTX in the R15 cell is quite

different from what is observed in the β -cell (6), we believe that the evidence for the importance of Ca^{+2} regulation in the β -cell is compelling. In the model given below, the differences between neuronal and β -cell oscillations are due primarily to the appearance of an inward Ca^{+2} current in β -cells that replaces the inward Na^{+} current of the neuron (7).

Our minimal model for the β -cell burst oscillations contains the four features cited by Atwater et al. (1). Explicitly we assume the following:

(a) A potassium channel activated by intracellular calcium ions and sensitive to quinine. We have adopted a modification of the scheme proposed by Plant (12), since the mechanism involved in the calcium activation is not known at present. The conductance of these channels is assumed to be

$$g_{K,Ca} = \bar{g}_{K,Ca} Y^{\ell} / (1 + Y^{\ell}) \quad (1)$$

where $\bar{g}_{K,Ca}$ is the maximum conductance per unit area, and $Y = \text{Ca}_i / K_{\text{dis}}$. Here K_{dis} is the dissociation constant for Ca^{+2} bound to the channel gate and ℓ is an integer. A value of $\ell = 1$ was chosen by Plant (12, 13), corresponding to noncooperative binding. We have used this value in most of our numerical work, except as described below. For K_{dis} we have taken the value of $1 \mu\text{M}$ suggested by Atwater et al. (1).

(b) A voltage-gated K^{+} channel sensitive to TEA. Following the idea set forth by Hodgkin and Huxley (7), the conductivity for this channel is written as

$$g_{K,HH} = \bar{g}_{K,HH} n^4, \quad (2)$$

where $\bar{g}_{K,HH}$ is the conductance per unit area when the channel is fully activated, and n is the fraction of K^{+} activation. In our work we have adopted the expressions for the time change of n as given by Hodgkin and Huxley. These equations depend on the variables n_{∞} , ϕ , τ_n , α_n , and β_n . In our model the voltage dependencies of these variables have the same form as the original Hodgkin-Huxley equations, but are shifted along the voltage axis by V^* , i.e., V is replaced by $V + V^*$. The value we have used for V^* is given in Table I.

Following the Hodgkin-Huxley scheme, the total current due to potassium channels is given by

$$I_K = g_K (V_K - V) \quad (3)$$

where V is the membrane potential, V_K is the resting potential for the K^{+} ion, and

$$g_K = g_{K,Ca} + g_{K,HH}. \quad (4)$$

(c) A voltage-gated calcium channel. We write the conductance of the voltage-dependent calcium channel in terms of the variables m and h

introduced by Hodgkin and Huxley (7). Thus

$$g_{Ca,HH} = \bar{g}_{Ca,HH} m^3 h. \quad (5)$$

m is the activation and h is the inactivation parameter of the channel. Again, to describe the time dependence of the activation and inactivation parameters, we adopt the usual Hodgkin-Huxley scheme. Thus, what is normally an inward sodium current in the Hodgkin-Huxley model becomes an inward Ca^{+2} current in our model. This is compatible with a variety of experimental facts, including the persistence of the bursting pattern in the presence of normally inactivating amounts of TTX (6). To describe the voltage dependence of time relaxation parameters α_m , α_h , β_m , and β_h we have used exactly the form given by Hodgkin and Huxley. However, the voltage V in α_m , α_h , β_m , and β_h has been replaced by $V + V^*$, with V^* given in Table I.

Given the conductance expression in Eq. 5, the calcium current is written

$$I_{Ca} = g_{Ca,HH} (V_{Ca} - V), \quad (6)$$

where V_{Ca} is the resting potential of calcium.

To reflect the occurrence of Na^{+} leaks (6) and, presumably, Cl^{-} leaks in the β -cell, we have added to our model a leak conductance. As in the Hodgkin-Huxley model, the current due to these leaks is written as

$$I_L = g_L (V_L - V). \quad (7)$$

In the Hodgkin-Huxley formalism, the membrane current is the sum of all the contributions from the above ionic channels. Thus, the time change of membrane potential may be expressed as

$$C_m dV/dt = g_K (V_K - V) + 2g_{Ca,HH} (V_{Ca} - V) + g_L (V_L - V) + I_{app} \quad (8)$$

where C_m is the membrane capacitance, and I_{app} is the applied external current.

(d) Glucose-activated loss of cytosolic Ca^{+2} . There are a variety of mechanisms that regulate the free calcium ion concentration, Ca_i , in the cytosol (14-16). These include a plasma-membrane bound Ca^{+2} ATPase that pumps Ca^{+2} out of the cell (14, 16, 17), a passive Ca^{+2} leak into the cell (8), the $\text{Na}^{+}/\text{Ca}^{+2}$ exchange mechanism (14, 17) mitochondrial uptake and release of Ca^{+2} (14, 15) and other fixed and mobile Ca^{+2} binding sites. Because little is known about these specific mechanisms in the β -cell, and because many details of these transport mechanisms are still unknown in other systems, we have chosen to model the metabolism of Ca^{+2} in a minimal fashion. We have included a single rate term for the change of Ca^{+2} in the cytosol of the form $-k_{Ca}\text{Ca}_i$. This specifically ignores the leak of external calcium ions into the cell, although this is probably justified by the slow rate of leakage (8).

Three of the Ca^{+2} transport mechanisms mentioned above contribute, in principle, to the efflux of Ca_i : the $\text{Na}^{+}/\text{Ca}^{+2}$ exchange, the mitochondrial uptake of Ca^{+2} , and the Ca^{+2} ATPase pump. The fact that burst oscillations in the β -cell occur only in the presence of glucose suggests that the efflux mechanisms is at least partially dominated by one that is coupled to glucose metabolism. While the activity of the Ca^{+2} ATPase depends explicitly on ATP, the K_M value of the ATPase for ATP is 0.05 mM (14), which is several orders of magnitude below physiological levels of ATP. Thus, an increase in glucose metabolism should be incapable of increasing the efflux of Ca^{+2} by this mechanism. Similarly, the $\text{Na}^{+}/\text{Ca}^{+2}$ exchange mechanism operates in the absence of ATP, although small amounts of ATP are known to stimulate Ca^{+2} binding to the presumptive carrier (14). This leaves mitochondrial uptake of Ca_i , a process that is known to be strongly coupled to the membrane potential of the mitochondria (14, 15), as the primary candidate for the glucose-activated efflux of Ca^{+2} . Indeed, recent experimental work (14), suggests that uptake occurs by a uniport mechanism that is stimulated by oxidative phosphorylation. Oxidative phosphorylation, of course, is coupled to

TABLE I
VALUES OF PARAMETERS IN THE MODEL

Parameter	Numerical value
$C_m (\mu\text{F}/\text{cm}^2)$	1
$\bar{g}_{K,Ca} (\text{mS}/\text{cm}^2)$	0.09
$\bar{g}_{K,HH} (\text{mS}/\text{cm}^2)$	12
$\bar{g}_{Ca,HH} (\text{mS}/\text{cm}^2)$	6.5
$g_L (\text{mS}/\text{cm}^2)$	0.04
$V_K (\text{mV})$	-75
$V_{Ca} (\text{mV})$	+100
$V_L (\text{mV})$	-40
$V^* (\text{mV})$	30
$V' (\text{mV})$	50
$K_{\text{dis}} (\mu\text{M})$	1
$r (\mu\text{m})$	8.9
f	0.004
$T (^{\circ}\text{C})$	20

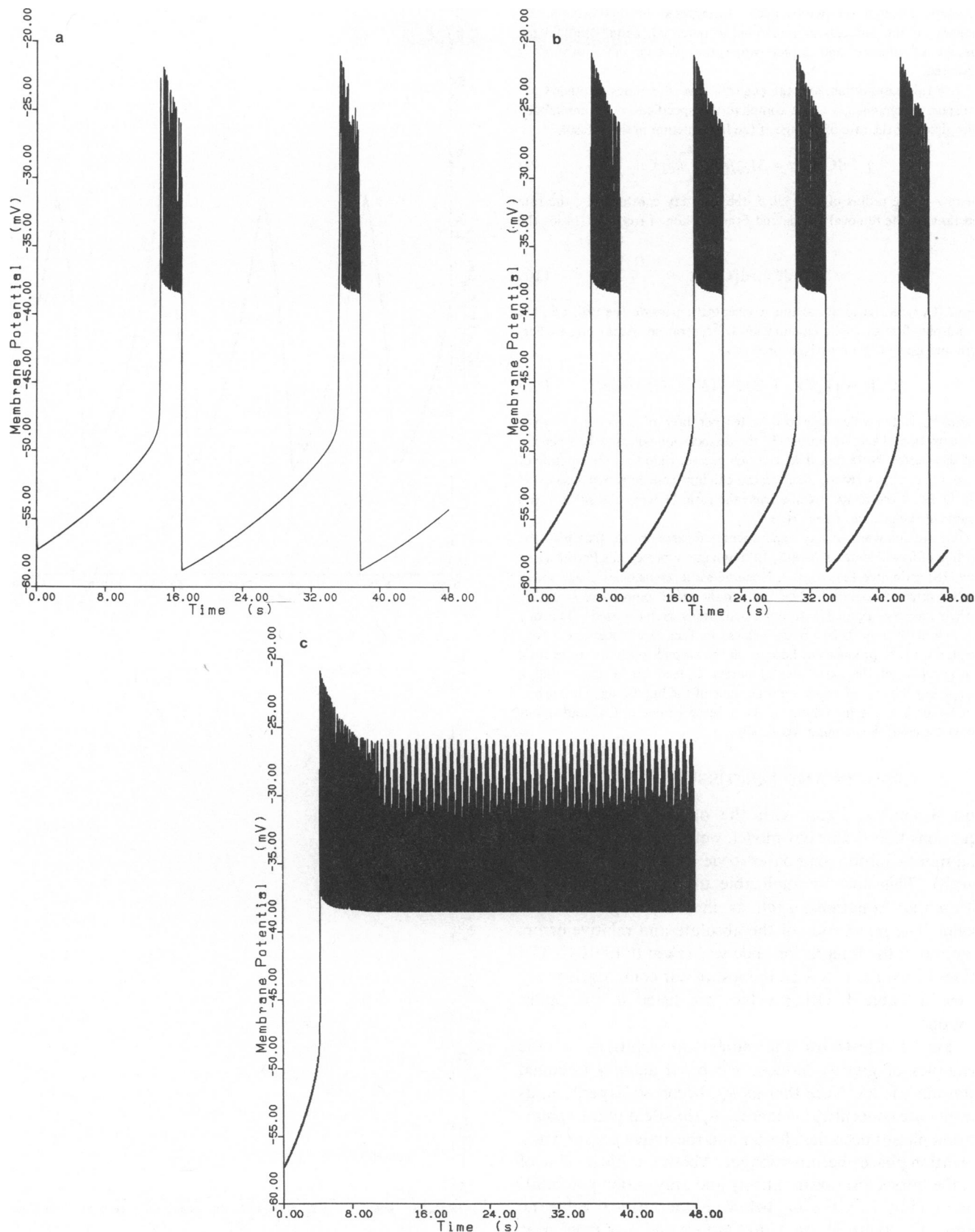


FIGURE 1 The burst pattern in variation of glucose. *a* was obtained with $k_{Ca} = 0.02 \text{ ms}^{-1}$, *b* with $k_{Ca} = 0.04 \text{ ms}^{-1}$, and *c* with $k_{Ca} = 0.06 \text{ ms}^{-1}$. This figure and the following three figures comes from calculations with $I_{app} = 0$. Initial conditions used in this figure and the following figures (with an exception of Fig. 4) are $V = -55 \text{ mV}$ and $Ca_i = 0.8 \mu\text{M}$. In Fig. 4 we set $Ca_i = 0.5 \mu\text{M}$ in order to facilitate the computation.

glycolysis through the Krebs cycle. Interpreted in this fashion, k_{Ca} depends on the mitochondria membrane potential, being small in the absence of glucose and larger when the glucose concentration is increased.

With this assumption, and the neglect of the dynamics of other Ca^{+2} transport mechanism, we can complete the specification of our model. This gives, for the rate of change of the free calcium in the cytosol,

$$f^{-1} dCa_i/dt = 3I_{Ca}/rF - k_{Ca} Ca_i \quad (9)$$

where r is the radius of the cell, F the Faraday constant, k_{Ca} the rate constant for the removal of Ca_i , and f the fraction of free Ca^{+2} inside the cell, i.e.,

$$f = dCa_i/d[Ca]_T \quad (10)$$

Here, $[Ca]_T$ is the total calcium concentration inside the cell, i.e., the bound-plus-free cytosolic calcium ion concentration. According to Ferreira and Lew (18) it may be expressed as

$$[Ca]_T = [V_w/V_c + \sum_j B_j/(K_j + Ca_i)] Ca_i \quad (11)$$

where V_w is the volume of cell water per liter of cells (V_c), B_j the concentration of the j th buffer, K_j the dissociation constant between Ca and this buffer. Note that if K_j is much greater than Ca_i , the fraction of ionized Ca^{+2} , f , is independent of the calcium concentration inside the cell. In Eq. 9 the factor 3 came from the ratio between the surface and volume of the cell, i.e., $(4\pi r^2)/(4\pi r^3/3)$.

We are unaware of any experimental measurements that give the fraction of free calcium in β -cells, although in red blood cells Ferreira and Lew (18) estimated that $f = 0.4$. Nonetheless it seems likely that a much smaller value will obtain in β -cells due to the more complicated structure of their internal organelles. In our calculations we have used values of f between 0.01 and 0.001. Such values, in fact, are necessary if our mechanism is to produce oscillations on the second-to-minute time scale characteristic of the experimental bursts. Indeed Eq. 9 shows that a change in f directly changes the time scale of the bursts, since it appears as a factor in the denominator of the time derivative of Ca_i , and in our model Ca_i controls the burst frequency.

RESULTS AND DISCUSSION

Eqs. 8 and 9, along with the other Hodgkin-Huxley equations that define our model, were integrated using the Shampine-Gordon computer code (University of Pittsburgh). This code is applicable to the solution of stiff differential equations, such as in the Hodgkin-Huxley model. The magnitude of the absolute and relative errors required in the integration code was taken to be 10^{-6} . The values of parameters used in most of our computations are given in Table I. Other values are listed in the figure captions.

Fig. 1 illustrates the numerical solutions of the dynamics of glucose-induced electrical activity obtained from our model. Note that as k_{Ca} becomes larger, i.e., as the glucose concentration increases, the silent phase (polarization phase) becomes shorter and the active phase (depolarization phase) becomes longer. Above a critical value of k_{Ca} the bursts disappear entirely and only action potentials remain (Fig. 1 c). For k_{Ca} below another critical value, no electrical activity of any kind appears and V is fixed near the resting potential of K^+ . This is in agreement with the experimental observation in β -cells (5).

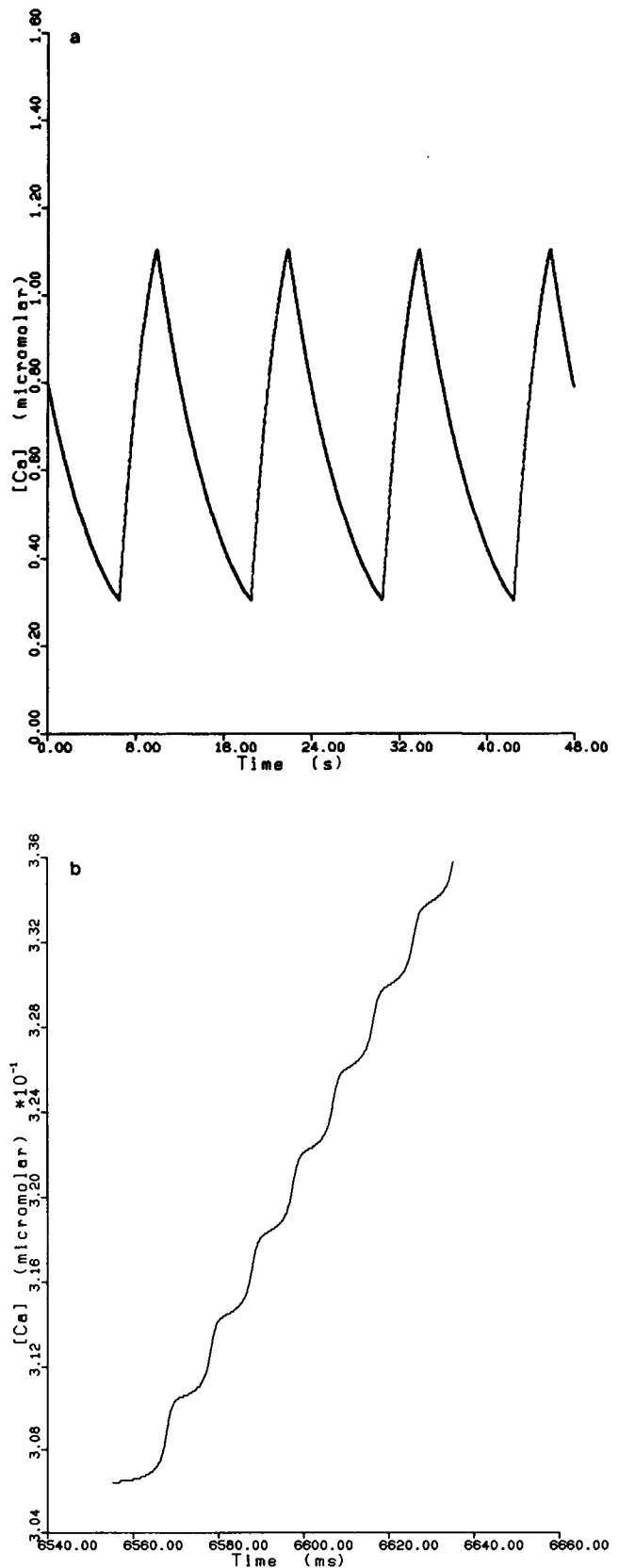


FIGURE 2 Variation of Ca_i with time. The conditions are the same as that of Fig. 1 b. b, the time scale is expanded by a factor of 500.

In Fig. 2 *a* we show the variation of calcium inside the cell with time, over a period of several oscillations. As expected, Ca_i continuously falls during the silent phase and rises abruptly during the active phase. On this time scale it is not possible to perceive the effect of individual action potentials during the active phase. This can be seen on an expanded time scale, as shown in Fig. 2 *b*. In that figure each action potential gives rise to a steplike increase in Ca_i .

Figs. 1 and 2 are compatible with the qualitative description of the oscillations given by Atwater et al. (1): In the absence of glucose, Ca_i is relatively high; thus, the permeability of K^+ is high and the membrane potential is at its most negative state. Upon addition of glucose, Ca_i is lowered by the activation of various energy-requiring mechanisms, probably the mitochondria. As Ca_i is lowered, the calcium-sensitive K^+ -channel becomes inhibited, inducing depolarization. When Ca_i is sufficiently reduced, a rapid depolarization to the plateau potential occurs; at the plateau potential, a series of action potentials occur due to the activation of the voltage-gated Ca^{2+} and K^+ channels (see Fig. 1). The inward current of Ca^{2+} results in an increase of Ca_i . After a volley of action potentials the calcium-sensitive K^+ -channel is activated, repolarizing the

membrane and inhibiting further influx of calcium. This inhibition leads to a decrease in Ca_i , and once the cell has managed to reduce Ca_i sufficiently the cycle starts again.

The repetitive action potential spikes at high glucose concentration (Fig. 1 *c*) are reminiscent of the repetitive firing of certain nerves stimulated by a fixed depolarizing current. Indeed, there is a great similarity between our description of glucose-induced spiking in the β -cell and Rinzel's treatment of repetitive neuronal activity (19). In our model, when k_{Ca} takes on a large enough value, the inward Ca^{2+} current is no longer sufficient to bring the Ca_i level up to a value that can reinstate the Ca^{2+} activated potassium current. Thus under high glucose a fixed part of the potassium current is always turned off. In the repetitive neuronal spikes, this circumstance is effectively achieved by the fixed depolarizing current.

The form of the burst patterns in Fig. 1 *a* and *b* is remarkably like those observed experimentally. Experiments with several hundred different β -cells (1) have shown a large variance in both the duration of the active (spiking) phase and the number of bursts per minute. In spite of this, the general features of the experimental oscillations are close to those we have found in our calculations. These features include such things as the minimum voltage (-57 mV), the overall amplitude of the

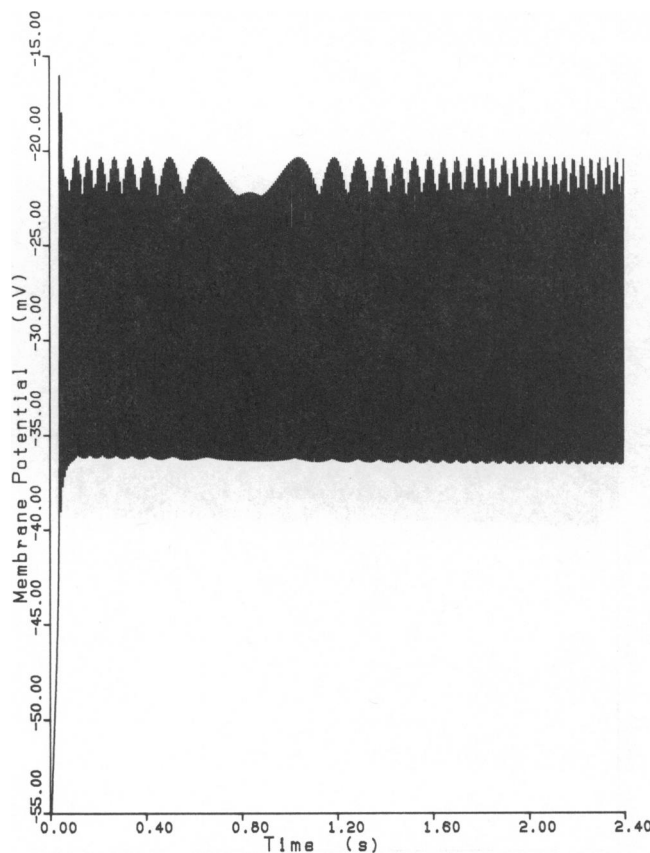


FIGURE 3 Effect of quinine on the burst pattern. In this calculation $k_{Ca} = 0.001 \text{ ms}^{-1}$ and $\bar{g}_{KCa} = 0.02 \text{ mS/cm}^2$.

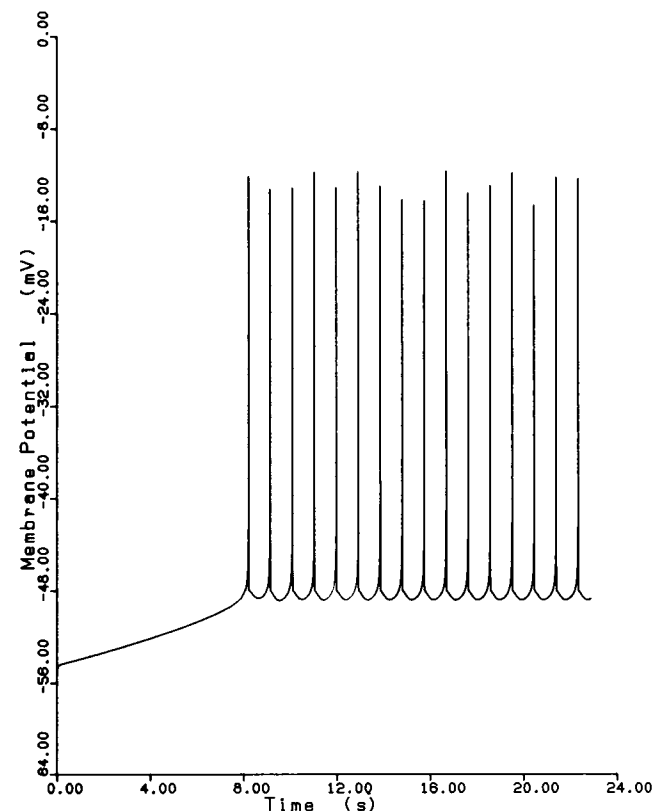


FIGURE 4 Effect of TEA on the membrane oscillation. In this calculation $k_{Ca} = 0.02 \text{ ms}^{-1}$ and the relaxation time τ_n was increased over that in Table I by a factor of 2.

oscillations (~ 30 mV), the average amplitude of the spikes (~ 12 mV), the irregularity of the action potentials with their tendency to decrease in amplitude during the active phase, the period of bursting (10–20 s), and the slow depolarization during the silent phase (see Fig. 1, reference 20). By changing the parameter values listed in Table I slightly, we are able to produce quantitative changes in the form of the oscillations that, nonetheless, retain the basic characteristics described above.

Quinine is known to block the Ca^{2+} activated K^{+} -channel, and thus to depolarize the silent phase leading to the generation of continuous activity and to the loss of the burst pattern (1). To mimic this behavior we have lowered the value of $\bar{g}_{\text{K,Ca}}$ from 0.09 to 0.02 mS/cm². The results for this smaller value of $\bar{g}_{\text{K,Ca}}$ are shown in Fig. 3. The rather complicated pattern of continuous spikes is similar to that found in the presence of 0.1 mM quinine and in the absence of glucose (1).

The β -cell membrane oscillations respond dramatically to TEA, which is known to block the voltage-dependent K^{+} channel by prolonging the outward K^{+} current (4). We have modeled this action of TEA in our calculations by increasing the value of the K^{+} outward-current activation time, τ_n , by a factor of 2. The resulting oscillations are shown in Fig. 4. Note that this abolishes the burst pattern and blocks the repolarization phase of action potentials.

Also, note that the amplitude of the action potential is significantly larger than in Fig. 1 *a* and that the potential at the foot of each spike is close to the potential during the silent phase in Fig. 1 *a*. This is in agreement with experimental observations (4).

It should be noted that our model does not explicitly consider effects due to the electrogenic $\text{Na}^{+}/\text{K}^{+}$ pump. The importance of the pump on the burst pattern, however, has been shown experimentally by Atwater and Meissner (21) and Meissner and Preissler (6). Nonetheless, when K^{+} or Na^{+} are removed from the external bathing solution, it takes a very long time until a significant change in intracellular K^{+} or Na^{+} is observed. Similarly, it takes up to 20 min after the $\text{Na}^{+}/\text{K}^{+}$ pump is blocked with ouabain to observe significant effects on the β -cell oscillation. Thus we may partially include the effect of the pump in our model by the following device: When the $\text{Na}^{+}/\text{K}^{+}$ pump is fully activated, the cytosolic Na^{+} and K^{+} ion concentrations change very little, and hence the outward current that is contributed by the pump varies very little with time. In other words, the current due to the pump acts effectively like an applied current (cf. I_{app} in Eq. 7) with a negative value. In Figs. 1–4 we have taken I_{app} equal to zero. However, as shown in Fig. 5 *a*, the presence of a constant negative current $I_{\text{app}} = -0.6 \mu\text{A}/\text{cm}^2$ changes our results qualitatively very little from those in Fig. 1. However, to

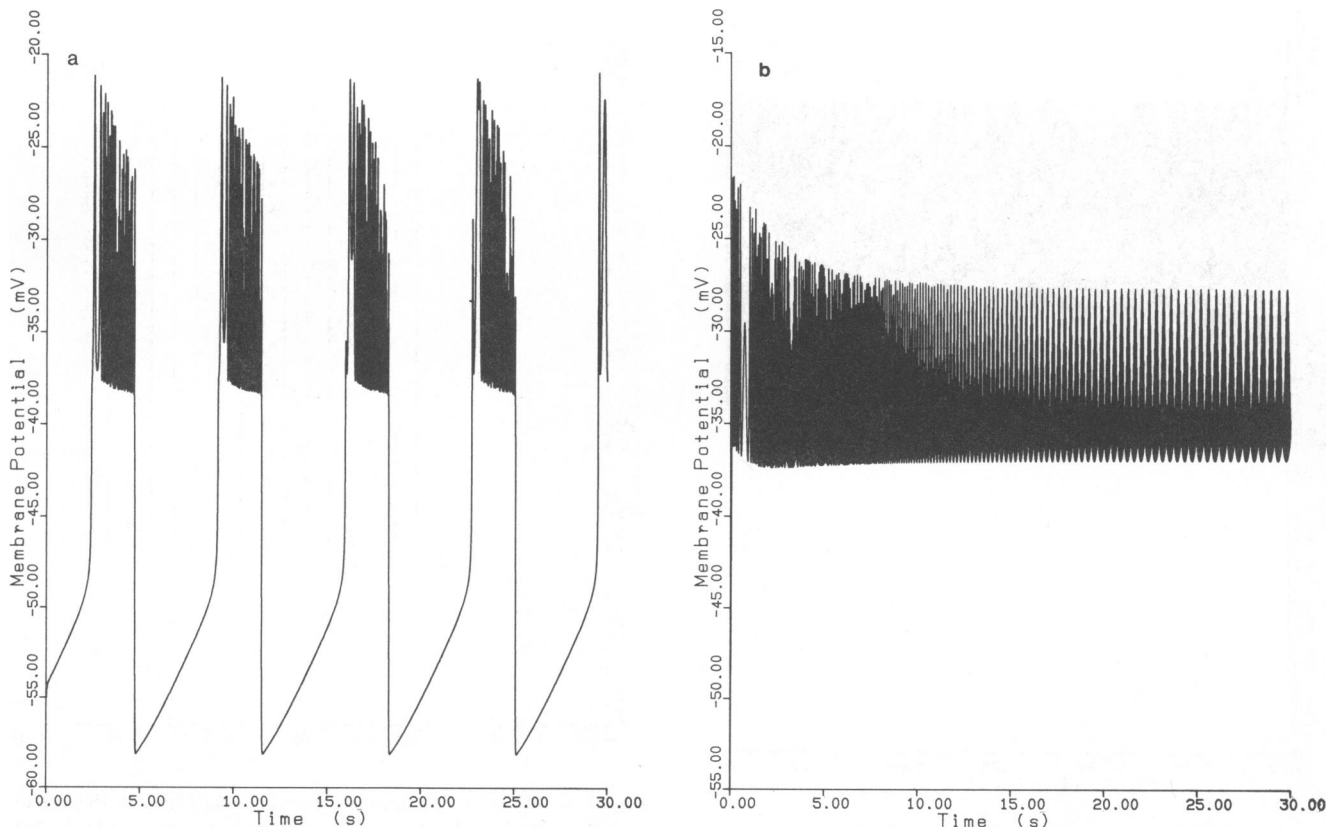


FIGURE 5 Effects of ouabain on the bursting pattern. Here $k_{\text{Ca}} = 0.084 \text{ ms}^{-1}$, $\bar{g}_{\text{K,Ca}} = 0.05 \text{ mS}/\text{cm}^2$, and $g_{\text{L}} = 0.06 \text{ mS}/\text{cm}^2$. *a* was obtained with $I_{\text{app}} = -0.6 \mu\text{A}/\text{cm}^2$, and *b* was obtained with $I_{\text{app}} = 0$. Calcium binding in Eq. 1 was taken as very cooperative, i.e., $\ell = 4$.

achieve the results in Fig. 5 *a* we have needed to use a smaller value of $\bar{g}_{K,Ca}$, a larger value of g_L , and to assume a Hill coefficient of $\ell = 4$ for the Ca^{+2} binding to the Ca^{+2} activated K^+ channel. These changes are shown in the legend to Fig. 5.

Using this slight extension of our minimal model, we can simulate the effect of the Na^+/K^+ pump inhibitor, ouabain, on the oscillations. Because ouabain shuts off the pump, we merely set $I_{app} = 0$. As shown in Fig. 5 *b*, this leads to continuous spiking. This is compatible with the observations of Atwater and Meissner (21) on the effect of ouabain. Another way of eliminating the action of the Na^+/K^+ pump is by reducing the external concentration of potassium to a low value. This is also known to produce a pattern of oscillations like that in Fig. 5 (1).

One of the striking experimental observations on the β -cell oscillations is the irregular appearance of the spikes in the active phase. We have also observed this irregularity in our numerical results, but only when they are plotted on the time scale of the overall oscillations. In Fig. 6 we show a graph of the same spikes plotted on a millisecond time scale. When plotted this way they are seen to be quite regular in shape and to have a gradually decreasing amplitude, as we would expect. Thus it would appear that much of the irregularity of the active phase spikes is an artifact of the time scale used to measure the oscillations. Another important aspect of the spikes shown in Fig. 6 is

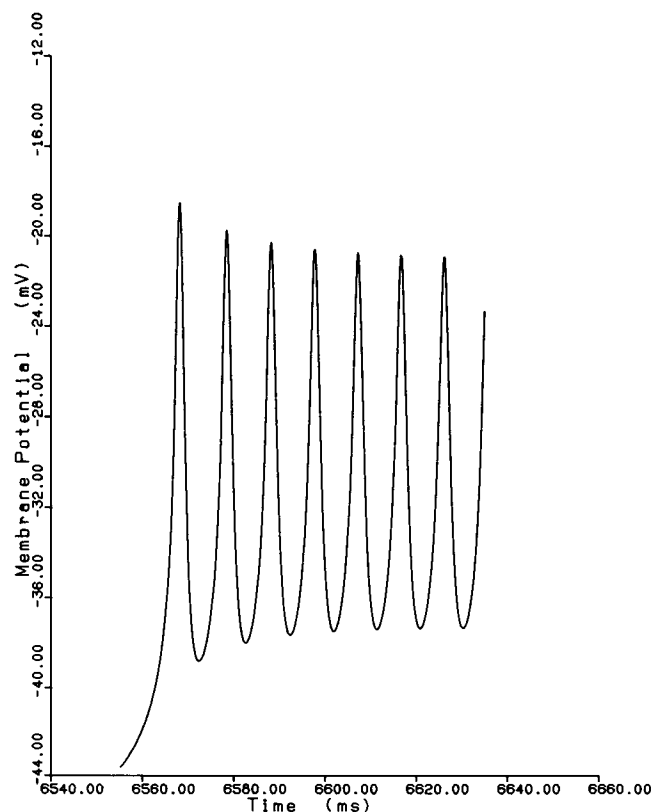


FIGURE 6 The action potential spikes shown in Fig. 1 *b*, but plotted on a time scale that is expanded by a factor of 500.

their period, which increases from 10 to 40 ms, and considerably shorter than the 50 ms period observed experimentally (20). This suggests to us that the parameters we have chosen for Hodgkin-Huxley conductances $g_{K,HH}$ and $g_{Ca,HH}$ are not correct. We can easily lengthen the duration of the action potentials by increasing the relaxation time constant for the calcium conductance in our modified Hodgkin-Huxley model, or by increasing the capacitance of the membrane slightly. However, any real improvement of this aspect of the model awaits the experimental determination of the Hodgkin-Huxley parameters for the β -cell.

SUMMARY AND CONCLUSIONS

Based on the experimental work of Atwater et al. (1), we have developed a minimal mathematical model to describe burst oscillations in the β -cell. The model is minimal in that it includes only the basic set of processes that lead to burst oscillations: voltage-regulated K^+ channels, Ca^{+2} -activated K^+ channels, voltage-regulated Ca^{+2} channels, and glucose-stimulated efflux of Ca^{+2} from the cytosol. With these basic processes the model produces burst oscillations with features like those observed experimentally. The response of the oscillations in our model to the action of glucose, quinine, or TEA is similar to that elicited experimentally.

In its minimal form our model is not capable of describing the effect on the oscillations of changing external concentrations of Na^+ or K^+ . To properly treat the effects of externally applied Na^+ and K^+ requires that the Na^+/K^+ pump be added to our model. Although we have not done that here, we have assessed the effect of turning off the pump using ouabain or low external K^+ concentrations. To do this we have extended the minimal model by including a fixed polarizing current to mimic the effect of the pump. Reducing the current to zero in this extended model gives a continuous volley of action potentials as is seen experimentally in the presence of ouabain (21).

One of the purposes of constructing a minimal model like ours is to assess which experimental facts are crucial to the oscillations. Based on our results, the Ca^{+2} activated K^+ channel appears to be the trigger for the oscillations (1, 5). The trigger is initiated by glucose which, in our model, acts by stimulating efflux of Ca^{+2} , probably into the mitochondria. While other Ca^{+2} transport mechanisms, such as the Na^+/Ca^{+2} exchange mechanism and the Ca^{+2} ATPase, are undoubtedly important in the β -cell, they do not appear to be strongly enough coupled to glucose metabolism to initiate oscillations. The Na^+/K^+ pump, on the other hand, has been implicated in the burst oscillations by several experiments. Our numerical work suggests that the pump is, indeed, important in moderating the membrane potential of the β -cell. In this way both external Na^+ and K^+ are coupled to the Ca^{+2} triggering mechanism. Our work, however, suggests that the Na^+/K^+ pump is not a crucial dynamical part of the oscillatory mechanism.

Calcium occupies a central control position in our model. Nonetheless its effect has been observed in experiments only indirectly. Based on our model, there are two aspects of the control of calcium that it may be possible to observe experimentally. First, as yet there has been no direct observation of internal calcium changes within the cell. Our calculations predict a calcium oscillation of the same frequency as the burst oscillations (Fig. 2 *a*) with a steplike fine structure during the active phase. Although the internal calcium concentration is quite small, it may be possible to measure these changes spectroscopically with Ca^{+2} -sensitive fluorescence probes or a Ca^{+2} -selective microelectrode. Another prediction, based on the way in which calcium enters our model, is that oscillations will cease if the calcium conductance is specifically blocked. This follows from the fact that, at fixed glucose concentration, the only mechanism in our model for reentry of calcium into the cell is through the Ca^{+2} conductance. If the Ca^{+2} conductance is blocked, no inward current of Ca^{+2} is possible, and only a steady depolarization of the membrane should be observed. This, of course, neglects the effect of other calcium transport mechanisms, such as the $\text{Na}^{+}/\text{Ca}^{+2}$ exchange mechanism and the Ca^{+2} ATPase. However, it seems unlikely to us that their effect could lead to anything but a slightly increased, but steady, value of Ca_i .

The blocking of the inward Ca^{+2} current in our model has a much different effect than the blockage of the inward (Na^{+}) current in the *Aplysia* R-15 neuron (9, 10). As Plant has shown, this leads to a simple slow wave, which can be thought of as an oscillation that underlies the observed burst pattern. In our model, the inward calcium current is absolutely essential to achieving any kind of oscillations. Nonetheless, there is a kind of slow-wave oscillation that can be generated with our model. It requires increasing the capacitance of the membrane from 1 to $5 \mu\text{F}$ and is shown in Fig. 7. Under these conditions the time scale of the action potentials is greatly lengthened; the gradual repolarization in the -30 mV region of that figure represents a single extended action potential that, by itself, is sufficient to increase Ca_i and reinstate the calcium-regulated potassium channel.

There is another way in which our model can generate a slow wave. If the Hodgkin-Huxley potassium conductance, $\bar{g}_{\text{K,HH}}$, is set equal to zero and the Hill coefficient, ℓ , for Ca in Eq. 1 is set equal to 10, a definite slow-wave oscillation is observed. On the other hand, if we take $\ell = 1$, as in Figs 1–4, and set $\bar{g}_{\text{K,HH}} = 0$, we find simply a steady depolarization of the membrane potential. Because it is doubtful that the calcium-activated potassium channel has a Hill coefficient as high as 10, our prediction is that a selective blockage of $\bar{g}_{\text{K,HH}}$ would lead only to a new steady value of the resting potential. Thus a slow wave, while built into our model under certain extreme conditions, does not appear to be an intrinsic part of the β -cell oscillations.

Ours is not the first dynamical model of the β -cell.

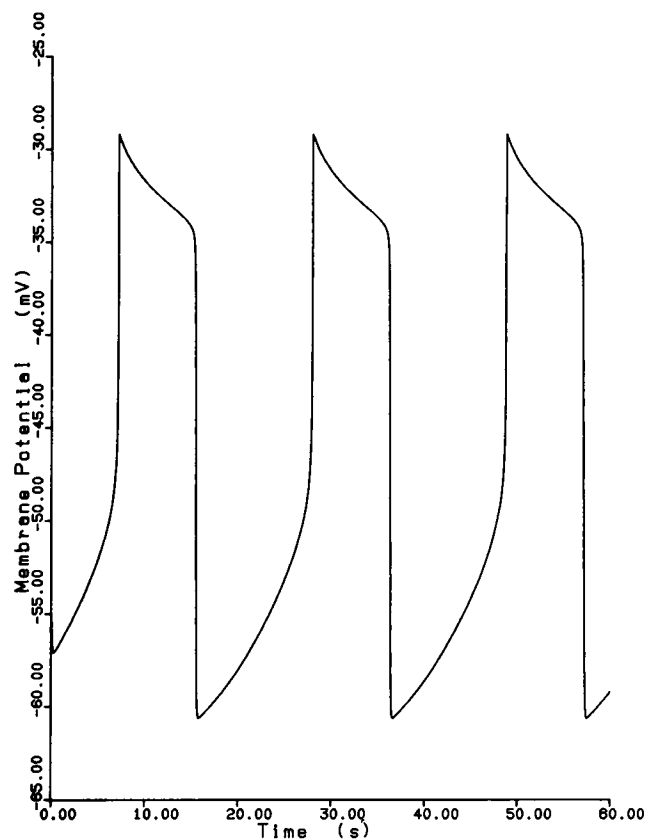


FIGURE 7 A burst-only type oscillation that occurs when the membrane capacitance is increased to $5 \mu\text{F}/\text{cm}^2$; all other parameters are as for Fig. 1 *b*.

Recently Matthews and O'Connor (22) have proposed a model based on the Goldman equation, extended to include divalent ions. Their model includes three potassium permeabilities, one of which is directly activated by glucose, rather than by calcium, as we have assumed. The Matthews-O'Connor model is a good deal more complicated than ours (some 46 parameters and variables are used) and is constructed with operational thresholds for most of the important events. For example, termination of the active phase is achieved in their model when an assigned threshold in the voltage is achieved. The similar event in our model occurs continuously as calcium reenters the cell and gradually increases the conductance, $g_{\text{K,Ca}}$, of potassium. Two other important differences between the models are that Matthews and O'Connor (*a*) include a changing internal concentration of potassium (K_i is constant in our minimal model) and (*b*) assume steady-state kinetics for Ca_i . Thus Ca_i is determined by permeability ratios, instead of a kinetic equation, like Eq. 9, that we use. Nonetheless the Matthews-O'Connor model is successful in producing burst oscillations. The chief advantages of our minimal model seem to be its simplicity and its direct connection with the Ca^{+2} activated K^{+} channel.

Our model needs to be extended to include the moderating effect of external calcium. Because large changes in

calcium are involved (20, 23, 24), large changes in the calcium resting potential occur. This means that a linearization around a fixed resting potential, as used in the usual Hodgkin-Huxley model, is not appropriate. Furthermore, we have determined that the Nernstian dependence of the calcium resting potential on Ca_i/Ca_e is not sufficient to reproduce the large effects observed experimentally. Consequently a dependence of the calcium conductance $\bar{g}_{\text{Ca,HH}}$ on external calcium must be introduced. This can be done systematically using the mechanistic theory of irreversible thermodynamics (25). Preliminary considerations suggest that this modification of the model will lead to agreement with experiments on the effect of changes in external Ca^{+2} concentration.

The authors wish to thank Professor R. E. Plant for several helpful conversations about his work.

Dr. Chay was supported by National Science Foundation grant PCM79-22483. Dr. Keizer was supported by Public Health Service grant GM30688-01 and National Science Foundation grant CHE 80-09816.

Received for publication 29 September 1982 and in final form 3 January 1983.

REFERENCES

1. Atwater, I., C. M. Dawson, A. Scott, G. Eddlestone, E. Rojas. 1980. The nature of the oscillatory behavior in electrical activity for pancreatic β -cell. In *Biochemistry Biophysics of the Pancreatic- β -Cell*. Georg Thieme Verlag, New York. 100-107.
2. Dean, P. M., E. K. Mathews, and Y. Sakamoto. 1975. Pancreatic islet cells: effects of monosaccharides, glycolytic intermediates and metabolic inhibitors on membrane potential and electrical activity. *J. Physiol. (Lond.)*. 246:459-478.
3. Atwater, I., B. Ribalet, and E. Rojas. 1978. Cyclic changes in potential and resistance of the β -cell membrane induced by glucose in islets of Langerhans from mouse. *J. Physiol. (Lond.)*. 278:117-139.
4. Atwater, I., B. Ribalet, and E. Rojas. 1979. Mouse pancreatic cells: tetraethyl blockage of the potassium permeability increase induced by depolarization. *J. Physiol. (Lond.)*. 288:561-574.
5. Atwater, I., C. M. Dawson, B. Ribalet, and E. Rojas. 1979. Potassium permeability activated by intracellular calcium ion concentration in the pancreatic β -cell. *J. Physiol. (Lond.)*. 288:575-588.
6. Meissner, H. P., and M. Preissler. 1980. Ionic mechanisms of the glucose-induced membrane potential changes in β -cells. In *Biochemistry Biophysics of the Pancreatic- β -Cell*. Georg Thieme Verlag, New York. 91-99.
7. Hodgkin, A. L., and A. F. Huxley. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.)*. 117:500-544.
8. Lew, V. L., and H. G. Ferreira. 1978. Calcium transport and the properties of a calcium-activated potassium channel in red cell membranes. *Curr. Top. Membr. Transp.* 10:217-277.
9. Junge, D., and C. L. Stephens. 1973. Cyclic variation of potassium conductance in a burst generating neuron in *Aplysia*. *J. Physiol. (Lond.)*. 235:155.
10. Levitan, I. B., A. J. Harmar, and W. B. Adams. 1979. Synaptic and hormonal modulation of a neuronal oscillator: a search for molecular mechanisms. *J. Exp. Biol.* 81:131-151.
11. Plant, R. E., and M. Kim. 1976. Mathematical description of a bursting pacemaker neuron by a modification of the Hodgkin-Huxley equations. *Biophys. J.* 16:227-244.
12. Plant, R. E. 1978. The effects of calcium⁺⁺ on bursting neurons. *Biophys. J.* 21:217-237.
13. Plant, R. E. 1981. Bifurcation and resonance in a model for bursting nerve cells. *J. Math. Biol.* 11:15-32.
14. Carafoli, E., and M. Crompton. 1978. The regulation of intracellular calcium. *Curr. Top. Membr. Transp.* 10:151-215.
15. Bygrave, F. L. 1978. Mitochondria and the control of intracellular calcium. *Biol. Rev. Cambr. Philos. Soc.* 53:43-79.
16. Schatzmann, H. J. 1975. Active calcium transport and the Ca^{+2} -activated ATPase in human red cells. *Curr. Top. Membr. Transp.* 6:125-168.
17. Blaustein, M. P. 1976. The ins and outs of calcium transport in squid axons: internal and external ion activation of calcium efflux. *Fed. Proc.* 35:2574-2578.
18. Ferrerira, H. G., and V. L. Lew. 1976. Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca. *Nature (Lond.)*. 259:47-49.
19. Rinzel, J. 1978. On repetitive activity in nerve. *Fed. Proc.* 37:2793-2802.
20. Ribalet, B., and P. M. Beigelman. 1979. Cyclic variation of K^{+} conductance in pancreatic β -cells: Ca^{2+} and voltage dependence. *Am. J. Physiol.* 237:C137-C146.
21. Atwater, I., and H. P. Meissner. 1975. Electronic sodium pump in β -cells of islets of Langerhans. *J. Physiol. (Lond.)*. 247:56-58P.
22. Mathews, E. K., and M. D. L. O'Connor. 1979. Dynamic oscillations in the membrane potential of pancreatic islet cells. *J. Exp. Biol.* 81:75-91.
23. Atwater, I. 1980. Control mechanisms for glucose induced changes in the membrane potential of mouse pancreatic β -cell. *Ciênc. Biol. (Portugal)*. 5:299-314.
24. Ribalet, B., and P. M. Beigelman. 1980. Calcium action potentials and potassium permeability activation in pancreatic β -cells. *Am. J. Physiol.* 239:C124-C133.
25. Keizer, J. 1976. Dissipation and fluctuations in nonequilibrium thermodynamics. *J. Chem. Phys.* 64:1679-1687.